Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 11718–11722, December 1993
Immunology

Age-dependent appearance of non-major histocompatibility complex-restricted helper T cells

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Communicated by Bernard Horecker, May 17, 1993

ABSTRACT **T cells which recognize antigen in association with self major histocompatibility complex (MHC) molecules are positively selected within the thymus. This results in skewing of the T-cell repertoire toward the recognition of antigenic peptides presented by self MHC molecules. While the thymus gland involutes at a relatively young age, bone marrow function and the size of the peripheral T-cell pool are maintained with age. We have investigated the MHC restriction of helper T-cell function for B-cell production of specific antibody in mice of different ages. Splenic helper T cells from 2- to 3-month old mice immunized with phosphocholine-keyhole limpet hemocyanin conjugate were MHC-restricted as defined by their capacity to induce phosphocholine-specific antibody synthesis by syngeneic but not by allogeneic B cells. In contrast, splenic T cells from immunized 18- to 20-month-old mice induced specific anti-phosphocholine antibodies by both syngeneic and allogeneic B cells. No evidence of polyclonal immunoglobulin synthesis was detected. The ability of T cells from old mice immunized with phosphocholine-keyhole limpet hemocyanin to induce phosphocholine-specific antibody synthesis by B cells from allogeneic mice was inhibited by T cells from immunized young mice. These findings suggest that non-MHC-restricted T-cell helper activity in old mice results from the loss of T cells, present in young mice, which suppress non-MHC-restricted helper cells.**

The aging of the immune system is characterized not only by decreased humoral (1) and cell-mediated (2) immunity to many foreign antigens but also by an increased production of autoantibodies (3) and monoclonal immunoglobulins (4). For this reason, we believe that immune senescence is an immune-disregulated and not merely an immune-deficient state. Age-associated thymic involution is thought to contribute to immune senescence.

The thymus gland is responsible for the development and shaping of the T-cell repertoire (5). Positive T-cell selection (6–7) skews the T-cell repertoire toward T cells that are activated by peptides derived from nominal antigens recognized in the context of syngeneic major histocompatibility complex (MHC) molecules. We asked whether that age-associated thymic involution would be associated with an alteration in the positive selection of T cells leading to degeneracy of self MHC restriction. Specifically, we wanted to know whether T cells from old mice immunized with phosphocholine-keyhole limpet hemocyanin (PC-KLH), a T-cell-dependent antigen, would induce anti-PC antibody synthesis by allogeneic B cells as well as syngeneic B cells.

MATERIALS AND METHODS

Animals. Young (6–8 weeks of age) BALB/c, C3H/HeJ, and C57BL/6 mice and adult (6 months) and old (18–22 months) BALB/c and C57BL/6 mice were purchased from the National Institute on Aging colony maintained by the Charles River Breeding Laboratories. Mice were maintained in our animal facility under laminar flow conditions.

Antigen Preparation. *p*-Aminophenylphosphocholine (Sigma) was diazotized to produce *p*-diazophenylphosphocholine (DPPC) (8). Ten milligrams of *p*-aminophenylphosphocholine was dissolved in 1.1 ml of 0.1 M HCl at 15–18°C. Three milligrams of $NaNO_2$ was dissolved in the above solution, and the pH was adjusted to 1–2. The DPPC was also conjugated to KLH (Calbiochem, La Jolla, CA) (9) to make PC-KLH. Ten milligrams of DPPC was added to 100 mg of KLH in 4 ml of phosphate-buffered saline (154 mM NaCl/8.1 mM Na_2HPO_4/1.9 mM NaH_2PO_4) at pH 8.9 on ice. The mixture was stirred for 18 hr at 4°C, dialyzed, and passed through a Sephadex G-50 column. DPPC was conjugated to sheep erythrocytes (Crane Laboratories, Syracuse, NY). The erythrocytes were washed three times in phosphate-buffered saline (pH 7.2) and twice in borate-buffered saline (0.07 M NaCl/0.1 M sodium borate, pH 8.9) and suspended at 20% (vol/vol) in borate-buffered saline. DPPC was added at a ratio of 0.02 ml per ml of erythrocytes. The erythrocytes were maintained at 25°C for 15 min and then diluted to 14% with borate-buffered saline for use in the plaque assay. Ten milligrams of KLH was dialyzed against borate-buffered saline overnight and the protein concentration was adjusted to 20 mg/ml. The KLH was centrifuged at 13,000 × *g* for 20 min and the supernatant was collected. A 15-mg/ml solution of 2,4,6-trinitrobenzenesulfonic acid (Sigma) in borate-buffered saline was made and was added to the KLH at a ratio of 38 μg of trinitrobenzenesulfonate per mg of KLH. The solution was protected from light and mixed at room temperature for 2 hr and then passed through an AG1-X8 resin column (Bio-Rad). The solution was dialyzed in phosphate-buffered saline (pH 8.9) for 2 days at 4°C and centrifuged at 13,000 × *g* for 20 min. The supernatant was collected and used to immunize mice.

Immunization. Young (4–8 weeks), adult (6 months), and old (18–22 months) BALB/c and C57BL/6 female mice were injected intraperitoneally with 20 μg of PC-KLH in 0.3 ml of complete Freund's adjuvant on days 0, 7, 32, and 39 (10).

Plaque Assay. One week after the last injection, mice were killed by cervical dislocation. Splenocytes were obtained from each mouse and T cells were isolated by nylon-wool column separation. The purity of the T-cell population was determined by indirect immunofluorescence staining using the anti-CD3ε monoclonal antibody (mAb) 1452C11 (11). Adherent cells were recovered by washing the nylon-wool column with warm RPMI 1640 medium (Hazelton Laboratories, Herndon, VA) and were used as antigen-presenting cells (APCs). APCs were also obtained from naive young BALB/c, C3H/HeJ, and C57BL/6 mice. The T cells and APCs were mixed together (2 × 10^5 T cells in 0.2 ml and 8 ×

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Abbreviations: PC-KLH, phosphocholine-keyhole limpet hemocyanin; MHC, major histocompatibility complex; mAb, monoclonal antibody; TCR, T-cell receptor; PFC, plaque-forming cell; APC, antigen-presenting cell; DPPC, *p*-diazophenylphosphocholine.

10^5 APCs in 0.8 ml) in 1 ml of complete medium per well of a 24-well plate (Nunc). The complete medium used was RPMI 1640 with 10% fetal bovine serum (HyClone), 2 mM glutamine, 20 μM 2-mercaptoethanol, penicillin (100 units/ml), streptomycin (10 μg/ml), and 0.1% gentamicin. For *in vitro* immunization, PC-KLH was used at 40 μg/ml of complete medium. The cells were cultured at 37°C for 4 days. On day 4 the cells were assayed in a modified Jerne hemolytic plaque assay (12). Fifty milliliters of distilled water containing 0.5 mg of agarose (Seakem, Rockland, ME) was mixed with 50 ml of Hanks' medium (JRH Biosciences, Lenexa, KS), and 500 μl of the mixture was added per test tube in a 45°C water bath. Fifty microliters of sheep erythrocytes and 50 μl of T-cell/APC suspension were then added to each tube, washed twice in Hanks' medium, and resuspended in 0.2 ml of Hanks' medium. The contents of each tube were poured on a slide, and the slides were placed in an incubator at 37°C. After 1.5 hr, 1 ml of a 1:30 solution of guinea pig complement (Hazelton) was added. The slides were incubated again at 37°C for 90 min, and hemolytic plaques were counted.

T-Cell Subset Separation. T cells suspended at 10^7 cells per ml were diluted 1:1 (vol/vol) with culture supernatant of the hybridomas secreting the rat anti-mouse L3T4 mAb Gk1.5 or with the murine anti-mouse Ly2.2 hybridoma HO2.2. Hybridomas were obtained from the American Type Culture Collection (Rockville, MD). Cells were incubated with the mAbs on ice for 30 min. Cells were then washed twice and resuspended at 2×10^7 per ml, and magnetic antibody-coated beads (Dynal, Great Neck, NY) were added at 4:1 bead/cell ratio. The cells coated with the murine anti-Ly2.2 mAb were incubated at room temperature for 15 min, diluted to 6.5 ml in Hanks' medium, and then incubated for 5 min before separation with a magnet. The cells coated with the rat anti-mouse L3T4 mAb were incubated on ice for 20 min before separation with a magnet. The purity of the negatively selected T-cell populations was determined by cytofluorographic analysis of cells stained with anti-L3T4 and anti-Ly2.2 mAbs in an indirect immunofluorescence assay.

Flow Cytometric Analysis of Cell Surface Molecules. Expression of T-cell receptor (TCR), CD3, CD4, and CD8 molecules was determined by direct immunofluorescence (13). R-Phycoerythrin-conjugated hamster anti-mouse CD3ε and rat anti-mouse L3T4 (CD4) mAbs and fluorescein-conjugated hamster anti-mouse TCR β-chain variable region $V_\beta 3$, hamster anti-mouse TCR δ polypeptide, mouse anti-mouse $V_\beta 8.1/8.2$, and rat anti-mouse $V_\beta 11$ mAbs were purchased from PharMingen. Cells were analyzed on an EPICS V 752 flow cytometry system (Coulter). Cells were double-stained with phycoerythrin-labeled anti-CD3 or anti-CD4 mAb and fluorescein-labeled anti-V_β antibodies. Ten thousand cells were counted for each sample and fluorescence and scatter signals were recorded. Fluorescence histograms were generated on a logarithmic scale (255 channels representing 3 orders of magnitude of fluorescence intensity). Fluorescence profiles were analyzed with the QUADSTAT program (Coulter), and fluorescence intensity comparisons were made by using mean channel number for each histogram and were standardized by beads expressing known amounts of fluorescein fluorescence (Flow Cytometry Standards, Research Triangle Park, NC.).

Mixed Lymphocyte Culture. Cultures were established in triplicate in 96-well round-bottom microtiter plates (Falcon) with 10^5 responding T cells (unseparated T cells, $CD4^+$ or $CD8^+$) from BALB/c PC-KLH-immunized mice and 10^5 irradiated (3000 rads; 1 rad = 0.01 Gy) allogeneic C57BL/6 non-T spleen cells. Cells were cultured in RPMI 1640 containing penicillin (100 units/ml), streptomycin (100 μg/ml), 2 mM glutamine, and 10% heat-inactivated fetal bovine serum. Cultures were incubated for 4 days at 37°C in a 5% CO_2/95% humidified air environment. [^{3}H]Thymidine (2 MCi, New England Nuclear); incorporation was measured during the last 8 hr of culture.

Statistical Analysis. Logarithmic means and standard deviations were computed for plaque-assay data. Comparisons were performed with the nonparametric Kruskal–Wallis test. The analysis was performed with the SAS Institute NPAR1WAY procedure.

RESULTS

T Cells from Old Mice Immunized with PC-KLH Help Syngeneic and Allogeneic B Cells to Produce Anti-PC Antibodies. To assess the effect of age on MHC restriction of helper T cells, splenic T cells from old and young BALB/c mice immunized with PC-KLH were cultured with syngeneic or allogeneic C57BL/6 B cells from naive young mice in the presence or absence of PC-KLH. T cells from young BALB/c mice provided help for syngeneic but not allogeneic naive B cells cultured with PC-KLH (Table 1, Exp. A). In contrast, T cells from old PC-KLH-immunized BALB/c mice provided help for both BALB/c (syngeneic) and C57BL/6 (allogeneic) B cells in the presence of PC. The difference between the allogeneic responses induced by T cells from old mice as compared to young mice was statistically significant ($P < 0.04$). We found no decrease with age in the B-cell anti-PC plaque-forming cell (PFC) response, confirming a previous report (14).

Ability of T Cells from Old Mice to Provide Help Across H-2 Differences Is Not Limited to One Mouse Strain. The ability of T cells from old mice to help an allogeneic B-cell anti-PC response is not restricted to the BALB/c strain. T cells from young or old C57BL/6 mice immunized with PC-KLH were cultured with syngeneic or allogeneic BALB/c B cells from naive young mice. The results obtained (Table 1, Exp. B) were similar to those obtained with T cells from BALB/c mice except for a higher PFC response of BALB/c B cells. Thus, T cells from old but not young C57BL/6 mice immunized with PC-KLH provided help for allogeneic BALB/c B cells to produce PC-specific antibody response; this difference was statistically significant ($P < 0.04$).

T cells from old mice induced a non-MHC-restricted B-cell response that was 75% of that induced in syngeneic B cells. In contrast, T cells from young BALB/c mice immunized with PC-KLH cultured with allogeneic B cells induced a significantly lower response (<10% of the anti-PC specific antibody response by syngeneic naive B cells). T cells from both BALB/c and C57BL/6 old mice consistently induced a higher antibody response by allogeneic B cells than did T cells from young mice.

If the appearance of non-MHC-restricted T cells were related to thymic involution, then non-MHC-restricted T cells might appear in middle-aged mice, in whom the thymus

Table 1. T cells from old PC-KLH-immunized mice provide help for naive syngeneic and allogeneic B cells to produce anti-PC PFC

Exp.	Age of T-cell donor	Source: T cells	Source: B cells	PFCs per culture: log(mean ± SD) (*n*)	PFCs per culture: Geometric mean
A	Y	BALB/c	BALB/c	3.03 ± 0.22(4)	1072
	O	BALB/c	BALB/c	2.99 ± 0.20(4)	979
	Y	BALB/c	C57BL/6	2.18 ± 0.27(4)*	151
	O	BALB/c	C57BL/6	2.80 ± 0.29(4)	631
B	Y	C57BL/6	C57BL/6	2.87 ± 0.12(3)	741
	O	C57BL/6	C57BL/6	2.74 ± 0.32(3)	550
	Y	C57BL/6	BALB/c	1.60 ± 0.52(3)*	40
	O	C57BL/6	BALB/c	2.66 ± 0.05(3)	457

*$P < 0.04$ for the difference between allogeneic PFCs induced by T cells from young (Y) and old (O) PC-KLH-immunized mice.

had involuted. To measure the age at which non-MHC-restricted helper T cells appear we immunized 4- to 8-week-old, 6-month-old and 18- to 20-month-old BALB/c mice with PC-KLH and measured their T-cell helper activity for syngeneic and allogeneic B-cell response. T cells from 6-week-old mice (thymus glands at maximal size) provided MHC-restricted help for syngeneic but not for allogeneic B cells. Thus, young T cells stimulated allogeneic B cells to produce <15% of the response obtained with syngeneic B cells. In contrast, T cells from mice of 6 and 18–20 months of age provided allogeneic help which represented 75% of the help provided to syngeneic B cells. T cells from immunized old and young mice induced anti-PC antibodies only in the presence of PC-KLH in culture. There was no evidence of T cells inducing a polyclonal B-cell response as measured by immunoglobulin in the culture supernatants (data not shown). Further, we detected no evidence that the capacity of T cells from old mice to help allogeneic B cells to produce anti-PC antibodies reflected an increased alloreactivity of T cells from old compared to young mice. Neither unseparated nor $CD4^+$ T cells had greater alloreactivity than did such populations from young mice. Thus [^{3}H]thymidine incorporation by unseparated T cells from old mice (4601 ± 1502, cpm × 10^{-3}) was no higher than that of unseparated T cells from young (5438 ± 1966) mice. In fact, $CD4^+$ T cells from old mice showed a significantly lower response (2516 ± 831) to allogeneic C57BL/6 cells than did $CD4^+$ T cells from young mice (7507 ± 2032).

L3T4 T Cells from Old Mice Induce PC PFC Response by Syngeneic or Allogeneic B Cells. To determine which subset of T cells contributed to syngeneic and allogeneic B-cell responses, L3T4 (CD4) or Ly2 (CD8) T cells from PC-KLH-immunized young and old BALB/c mice were purified by negative selection using antibody-coated magnetic beads. L3T4- or Ly2.2-depleted T-cell populations were cultured in the presence of PC-KLH with syngeneic or allogeneic naive B cells. T-cell populations depleted of L3T4 cells from young or old BALB/c PC-KLH-immunized mice induced a very low PC PFC response by syngeneic B cells (young, 55 ± 7 PFCs per culture; old, 105 ± 134) compared with unseparated T cells (young, 745 ± 219; old, 1020 ± 311). Similarly, T cells depleted of L3T4 cells from old BALB/c immunized mice had little or no capacity to help PC PFC response by allogeneic C57BL/6 B cells (75 ± 99) compared with unseparated T cells (710 ± 395). Depletion of Ly2.2 T cells from young mice decreased only minimally their capacity to help a syngeneic B-cell PC PFC response. T cells from old mice depleted of Ly2 T cells partially reduced their capacity to help a PFC response by syngeneic and allogeneic B cells. T helper activity of such T cells from old mice was comparable for syngeneic and allogeneic B cells. Results of a representative experiment are shown in Fig. 1. Thus $CD4^+$ T cells from old mice provide help for syngeneic and allogeneic B cells to produce anti-PC PFCs.

T Cells from Young Mice Suppress Non-MHC-Restricted Helper Activity of T Cells from Old Mice. The non-MHC-restricted helper activity found in old mice may reflect the appearance of T-cell helper activity for allogeneic B cells in old animals or the absence of suppressor activity for such cells in old mice. To distinguish between these two possibilities, we cultured T cells from old naive BALB/c mice alone or together with T cells from young mice, with allogeneic B cells in the presence of PC-KLH. A representative experiment is shown in Fig. 2. T cells from old mice induced allogeneic B cells to produce anti-PC antibodies (430 PFCs per culture). The addition of T cells from young mice to T cells from old mice cultured with allogeneic B cells suppressed the anti-PC allogeneic response by 75% compared with that induced by T cells from old mice cultured with allogeneic B cells (100 PFCs per culture). To examine the

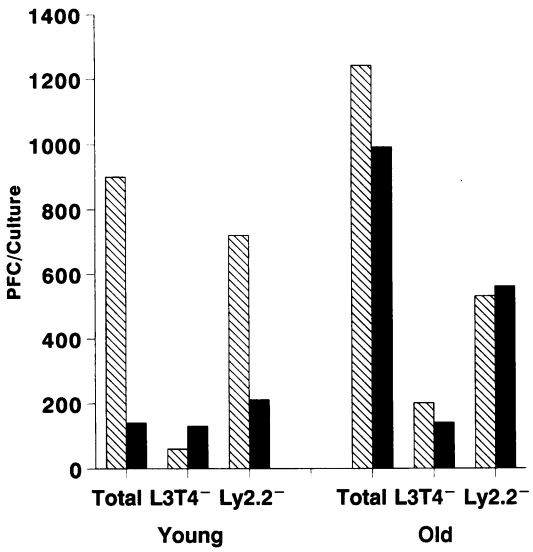

FIG. 1. Role of L3T4 and Ly2 T cells in syngeneic and allogeneic PC PFC responses. T cells from young and old PC-KLH-immunized BALB/c mice were depleted of $L3T4^+$ or $Ly2.2^+$ cells by antibody-coated magnetic beads and cultured in the presence of PC-KLH (40 μg/ml) and syngeneic (hatched bars) or allogeneic (filled bars) enriched naive B cells. After 4 days cultures were harvested for PFC assay against PC-coupled sheep erythrocytes.

phenotype of the young T cells that suppress allogeneic B cell responses, Ly2.2-depleted T cells from young mice were mixed with T cells from old mice and cultured with allogeneic B cells in the presence of PC-KLH. This population retained 75% of the response induced by the entire T-cell population from old mice alone (300 PFCs per culture). These results suggest that the suppressor activity for helper T cells resides primarily in the $Ly2.2^+$ T-cell population of young mice. The mixture of T cells from old and young donors was repeated four times with similar results.

Phenotypic Analysis of Spleen Cells from Old and Young Immunized Mice. Splenic cells from old and young mice had

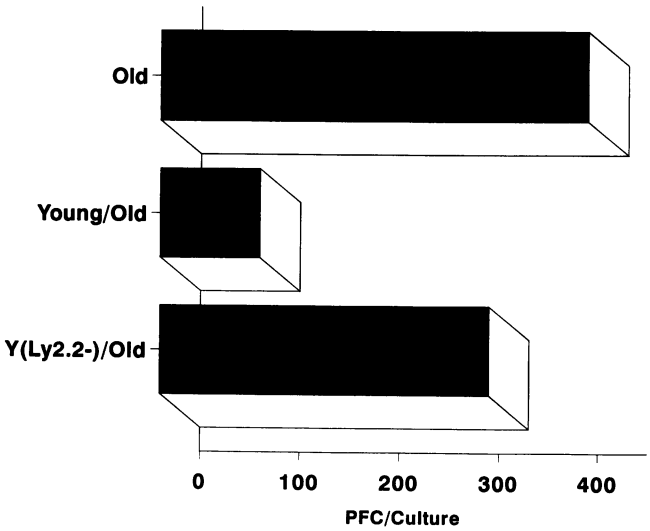

FIG. 2. T cells from young mice suppress PC PFC allogeneic response induced by T cells from old mice. T cells (10^5) from young naive BALB/c mice and T cells (10^5) from naive old BALB/c mice were cultured for 10 days together with syngeneic APCs in the presence of PC-KLH (40 μg/ml). Cultures were harvested, and activated T cells were cultured with allogeneic naive enriched B cells in the presence of PC-KLH. After 4 days cultures were harvested and assessed for PFC assay against PC-coupled sheep erythrocytes.

similar percentages of T cells bearing CD3, L3T4, Ly2, and the TCR $V_\beta 3$, $V_\beta 8.1/8.2$, $V_\beta 11$ or $\gamma\delta$ molecules (Table 2). Cells were stained both with anti-CD3ε R-phycoerythrin-conjugated mAb and with anti-L3T4, Ly2, $V_\beta 3$, $V_\beta 8.1/8.2$, $V_\beta 11$ or $\gamma\delta$ TCR fluorescein-conjugated mAbs. We chose to analyze $V_\beta 3$, $V_\beta 11$, and $V_\beta 8$ TCRs because in BALB/c mice the former two V_β types are negatively selected during differentiation in the thymus whereas the latter is not. As before (15), a greater percentage of $CD3^+$ cells was present in the spleen from young compared with old BALB/c and C57BL/6 mice. No significant difference in the percentage of L3T4 cells was observed between old and young mice. Spleens from young BALB/c mice had twice as many Ly2 T cells, as did spleens from old BALB/c mice, whereas similar numbers of Ly2 T cells were present in the spleens of young and old C57BL/6 mice. The cell surface density of Ly2 molecules was consistently higher in old than in young BALB/c and C57BL/6 mice. The numbers of $V_\beta 8.1/8.2^+$ cells were comparable in old and young BALB/c and C57BL/6 mice, although there was a slight increase in $V_\beta 3$ and -11 T cells in old BALB/c and old C57BL/6 mice. These results suggest that clonal deletion of these V_β genes in BALB/c mice is not complete. We also analyzed the percentage of $\gamma\delta$ TCRs because it has been reported that T cells expressing $\gamma\delta$ receptors are in general less MHC-restricted than their $\alpha\beta$-expressing counterparts and because they may be increased in aged mice. No difference in the number of $\gamma\delta$ T cells was observed in old and young BALB/c mice, while a slight but not significant increase in $\gamma\delta$ T cells was observed in old mice relative to young C57BL/6 mice.

DISCUSSION

Mature, $\alpha\beta$ TCR-bearing helper T cells in young mice normally recognize processed antigenic peptides presented by syngeneic but not allogeneic MHC class II molecules. For this reason, the cognate interaction between helper T cells and B cells which leads to antigen-specific antibody synthesis is termed MHC restriction. In contrast, T cells from mice over 6 months of age help allogeneic as well as syngeneic B cells to produce anti-PC antibodies. We have considered several mechanisms to explain this age-associated effect: (*i*) a decline in positive selection within the involuted thymus of mice over 6 months old; (*ii*) an increase in $\gamma\delta$ T-cell number or activity of these non-MHC-restricted cells; and (*iii*) a loss of cells which inhibit non-MHC-restricted T cells.

Table 2. Phenotypic characterization of spleen cells from young and old PC-KLH-immunized mice

mAb conjugate	Specificity	% positive cells			
		BALB/c		C57BL/6	
		Young	Old	Young	Old
500A2 PE	CD3	55.1	35.2 (85)	38.3	30.9 (78)
RM-4.5 FITC	L3T4	27.7	25.3 (111)	15.1	15.8 (135)
53-6.7 FITC	Ly2	17.8	7.9 (124)	15.3	17.8 (121)
KJ25 FITC	$V_\beta 3$	0.8	2.4 (103)	2.2	3.7 (108)
MR5.2 FITC	$V_\beta 8.1/8.2$	13.7	11.4 (91)	9.1	8.0 (95)
RR3-15 FITC	$V_\beta 11$	1.2	2.8 (82)	2.6	3.7 (100)
GL3 FITC	$\gamma\delta$	3.7	3.3 (78)	2.0	4.5 (105)

Spleen cells were assayed by direct immunofluorescence staining for T-cell subset markers and TCR V_β gene family usage. Cells were double-stained with anti-CD3ε R-phycoerythrin (PE)-conjugated mAb and with each of the fluorescein isothiocyanate (FITC)-conjugated mAbs. Data represent percent doubly stained cells. Values in parentheses give cell surface density for old relative to young in %. Comparison of fluorescence intensity was made by using mean channel number for each histogram and was standardized as described in *Materials and Methods*.

T-cell recognition across H-2 differences was observed in both old BALB/c and old C57BL/6 mice. As T-cell-depleted spleen cells from young mice were used as the source of B cells and APCs, the only variable studied was the age of the T-cell donor. The purity of the T-cell population, as determined by cytofluorography, was always >95%. The ability of T cells from old mice to help allogeneic B cells of different H-2 haplotype is due neither to polyclonal B-cell activation, as no polyclonal immunoglobulin production was induced, nor to an increase in allogeneic T-cell reactivity. In fact, helper T-cell activity has been reported to decrease with age (16).

While T-cell function is usually MHC-restricted, unorthodox T-cell help has also been described (17–21). Chimeric mice from high and low poly(Tyr,Glu)-poly(DL-Ala)-poly(Lys) responder strains produced specific antibody of low responder allotype (22). These results were interpreted as an indication of collaboration between histoincompatible high-responder T cells with low-responder B cells. Furthermore, T-cell clones have been described (23) which recognize pigeon cytochrome *c* presented by either A^k or A^s MHC molecules. However, it was not established whether T cells with altered MHC restriction were also self-reactive. In general, the ability of T cells to react across MHC differences has been described as "degeneracy" of MHC restriction and has been observed in a few isolated cases. Interestingly, this degeneracy of MHC restriction has been observed in long-term culture of T-cell clones or lines (24). The increased surface expression of CD3 and Ly2 molecules during prolonged culture has been suggested to be responsible for the observed degeneracy (25). We found no increase in the density of CD3 and Ly2 membrane expression on T cells from old compared with young mice. Further, the number of $Ly2^+$ cells was similar in old and young C57BL/6 mice and was actually lower in old BALB/c mice than in young BALB/c mice.

The observed T-helper function across MHC differences may reflect either the intrinsic quality of a particular TCR or a heterogeneity in T-cell populations of old mice. $\gamma\delta$ T cells, which constitute 1–10% of peripheral T cells, have been shown to be less MHC-restricted for antigen recognition than $\alpha\beta$ T cells (26, 27). Non-MHC-restricted $\gamma\delta$ T-cell hybridomas specific for purified mycobacterial protein derivative have been described (28). These hybridomas used a single γ-chain gene and a limited set of δ chains but showed high junctional diversity. It is possible that $\gamma\delta$ T cells from old mice may participate in non-MHC-restricted T help, although the percentage of $\gamma\delta$ T cells in old and young mice is comparable. In any case, and whatever the contribution of $\gamma\delta$ cells to non-MHC-restricted help is, in elderly humans some influenza-specific non-MHC-restricted T-cell clones express $\alpha\beta$ TCRs (29).

It is possible that non-MHC-restricted T–B cognate interaction in PC-specific antibody response by helper T cells relates to a recognition of antigen presented by relatively invariant cell surface molecules such as Qa or Tla or perhaps in association with a non-MHC molecule such as CD1. Murine T-cell clones and hybridomas recognizing epitopes on an MHC-linked gene distal to the *Q* region have been described (30, 31). Further, the TCR of a $\gamma\delta$ T-cell hybridoma was found to recognize a novel MHC *Tl* gene (32). The functional ability of these "non-classical" invariant MHC molecules to present foreign antigens was recently determined by the isolation of a helper T-cell hybridoma that recognizes a synthetic Glu-Tyr copolymer in association with Qa-1 molecules (33). In the human system, a putative role for a non-MHC antigen-presenting molecule has been proposed for CD1a, which has significant sequence homology to MHC class I molecules (34) and, like MHC class I molecules, can noncovalently associate with β_2-microglobulin (35) and can

be recognized by T cells (36). This hypothesis could be tested by obtaining MHC-identical APC lines which lack some of these monomorphic cell surface molecules and showing that T cells from old mice no longer respond to the antigen.

The non-MHC-restricted helper T-cell activity was suppressed by addition of T cells from 6- to 8-week-old mice. Addition of T cells from young mice did not inhibit the help for syngeneic B cells provided by T cells from old mice. These results suggest that young animals have a population of suppressor cells which inhibit T-cell help of allogeneic B cells. The effect of age on suppressor-cell activity is controversial. In general, nonspecific suppressor-cell activity has been found to increase with age, whereas specific suppressor T-cell activity has been found to increase or decrease with age. Thus, the generation of spontaneous $Ly2^+$ nonspecific suppressor cells (37) and of H-2 specific suppressor T cells (38) has been found to decrease in old C57BL/6 mice. The suppression exerted by T cells from young mice on helper T cells from old mice may depend upon an idiotype–anti-idiotype interaction. We (39) and others (40) have postulated that T–T cell idiotypic interactions participate in the maintenance of peripheral T-cell tolerance. In this study, we have found T cells in young immunized BALB/c mice that suppress the help across H-2 differences provided by T cells from old mice. Thus, the mechanism underlying the appearance of non-MHC-restricted T cells in old mice results from an age-related loss of $Ly2^+$ T cells that suppress T-cell help for allogeneic B cells. This may result from the involution of the thymus.

The functional significance of the presence of these non-MHC-restricted T cells in old individuals is not known. The fact that we have found an impaired response to influenza vaccination in elderly humans (41) and non-MHC-restricted influenza-specific T-cell clones (29) may suggest a role for these cells in the age-associated changes in immune response.

We acknowledge the helpful suggestions and the critical review of the work by Dr. J. G. Thorbecke. This work was supported in part by the National Institute on Aging (AG00541, AG08707). C.R. is the recipient of an Irma T. Hirschl/Monique Weill-Caulier Trusts Career Scientist Award. E.P.C. was supported by a National Institute on Aging training grant (AG00111).

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